Material and Methods: We used the EpCAM expressing squamous cell carcinoma cell lines Kyse 30 and 520 as in vitro model. To measure the potential effects of loss of EpCAM expression, we used the lentiviral pGIPZ shRNAmir system with two different sh-RNAs directed against EpCAM and one control shRNA vector. The EpCAM-suppression of the transduced cells was tested by quantitative RT-PCR and immunoblotting. Those cell lines with at least 80% reduction of EpCAM expression were further analysed. We used the "Fence-assay"® to investigate the migration. The tumour cell invasion was assessed with a commercially available Matrigel-coated Transwell system®. Transcriptome profiling of shRNA-transduced cell lines and control-vector transduced cell lines, respectively, was done with Agilents "*whole genome Array*".
Results: The migration of EpCAM-shRNA-transduced squamous cell carcinoma cells was reduced by 30−50% compared to tumour cells transduced with the control-vector. A 3−4% reduction of the invasion was observed. Both, the reduction in migration and invasion were statistically significant. Changes in the transcriptome expression were noted in shRNA-transduced cell lines compared to control-vector transduced cell lines. The differentially expressed genes fell in the categories cell structure, cell movement and developmental processes.
Conclusion: Our data indicate an active biological role of EpCAM in oesophageal squamous cell carcinoma progression and makes it a promising therapeutic target for this entity. However, the exact mechanisms of action warrant further investigation.

519 Impact of ECM on phenotype and EGFR inhibition in colorectal cancer cell lines

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Background: 3D tumour cell cultures grown in extracellular matrix (ECM) are considered to reflect human tumours more realistic than monolayers grown on plastic. ECM has not only drastic effects on phenotype but also on response to targeted therapies, as recently demonstrated in breast cancer cell lines. Here, we systematically investigated the impact of ECM on phenotype and on EGFR inhibition in commonly used colorectal cancer (CRC) cell lines.
Material and Methods: On-top matrigel assays were performed with SW480, HT29, DLD-1, LOVO, CACO, Colo 205 and Colo 206F cell lines. The phenotype of the 3D culture was assessed and compared to conventional 2D cell culture. Expression of genes involved in proliferation and cell adhesion was analysed on the transcriptional (quantitative RT-PCR) and on the protein level (immunoblotting and confocal imaging). Invasive capacity of the cell lines was assessed via Matrigel®-Boyden chamber assay. EGRF inhibition was achieved using tyrphostin AG 1478.
Results: A specific spheroid growth pattern was observed for all investigated CRC-cell lines. DLD-1 and CACO showed a clear solid tumour cell formation, HT29, SW480 and LOVO exhibited budding structures, while Colo 205 and Colo 206F showed grape-like structures. The 3D culture phenotype of the cell lines was not correlated to their invasive/migratory capacity. A significant reduction of the gene expression was noted for most investigated genes in 3D culture. In contrast, E-cadherin was up-regulated in several cell lines. Effects of EGFR inhibition was noted in 2D and 3D culture of sensitive cell lines.
Conclusion: The observed differences between the cell culture models corroborate the influence of ECM for cancer growth. Compared to conventional 2D cell culture, the 3D cell culture model (Matrigel® on-top assay) offers the opportunity to investigate potential molecular targets under more realistic conditions.

520 Effect of selenium on rat liver cell proliferation after partial hepatectomy

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Background: Studies on tumour cell lines, animal studies and human trials have demonstrated a tumour preventive effect of selenium (Se). Selenium-treatment in tumour preventive doses inhibit liver cell proliferation in both preneoplastic and neoplastic lesions in a rat liver carcinogenesis model. The selenium dependent redox-enzyme thioredoxin reductase (TxR1) was over expressed in proliferating nodular liver lesions in the model. In this work we have studied the effect of selenium on regenerative cell proliferation and on the expression of TrxR1 in rats after 2/3 partial hepatectomy (PH).
Material and Methods: Fischer344 male rats were given 5ppm sodium selenite in the drinking water one week prior to PH, and until sacrificed. Non-treated hepatectomised and non-treated sham operated rats were used as controls. Bodyweights and relative liver weight were monitored. Cell proliferation, mitotic figures and occurrence of TrxR1 were determined immunohistochemically (IHC). TrxR1 enzyme activity, mRNA expression, and protein levels were analysed using TrxR1-assay, real-time PCR and western blot.
Results: No differences in bodyweights, relative liver weights and regeneration of liver mass were shown between groups. The peak of the S-phase marker Mib5 coincided while the peak of the mitotic figures was slightly delayed in treated rats. IHC staining for TrxR1 revealed a zonal, periportal increase of enzyme expression at 24h post PH, corresponding to the zone of Mib 5 positive cells and mitotic figures. After PH the TrxR1 enzyme activity increased from 8 hours with a peak at 48 hours post PH in Se-treated animals. In non-treated animals a similar but lower induction of the activity was shown between 8−72 h post PH. The TrxR1 activity was not changed over time after sham-surgery. TrxR1 mRNA increase at 4 hours post PH was seen in all groups.
Conclusions: We have concluded that, although a slight delay of cell division was shown, the gain of liver mass and regeneration of the liver function after PH is not affected by selenite. The increase of thioredoxin reductase correlated with cell proliferation and was further induced by selenium.

521 Enhanced pulmonary tumourigenesis by N-nitrosobis (2-hydroxypropyl) amine after thoracic irradiation with X-rays in new born, juvenile and adult Wistar rats

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Background: The possibility that combined exposures to environmental pollutants and ionizing radiation could increase the risk of lung cancer of the general public is a matter of great concern. We investigated the combined effects of radiation and a chemical carcinogen on pulmonary tumourigenesis in rats exposed at different age in well-defined exposure conditions.
Material and Methods: Female 1-, 5- and 22-week-old Wistar rats were irradiated locally on the thorax with X-rays (3.18 Gy), and/or were given *N*-nitrosobis (2-hydroxypropyl) amine (BHP; 1 g/kg body weight) intraperitoneally 1 week after thoracic irradiation.
Results: Non-irradiated and non-BHP-injected control rats survived to 90 weeks of age when all rats were sacrificed, but administration of BHP with or without irradiation resulted in survival reduction due to kidney, brain, liver and ovarian tumours. The incidences of lung tumours including adenomas and adenocarcinomas in rats irradiated alone at 1, 5 and 22 weeks were 8.7, 15.0 and 20.0%, respectively. On the other hand, the incidences in the rats administered with BHP alone at 2, 6 and 23 weeks were 60.9, 25.0 and 30.0%, respectively. When a combination of irradiation and BHP was used, the incidences in the rats treated at 1−2, 5−6 and 22−23 weeks were 61.9, 65.0 and 55.0%, respectively. The incidence of adenocarcinomas in the rats treated at 5−6 weeks was significantly increased compared to rats exposed to either X-rays or BHP alone.
Conclusion: The combined effects are age-dependent and administration of BHP after X-ray irradiation synergistically enhances induction of lung adenocarcinomas in juvenile rats. These results indicate that Wistar rats exposed to X-rays and BHP are a suitable animal model to study the risk and the mechanisms of the combined effects of radiation and chemicals on pulmonary tumourigenesis.

522 Selenium homeostasis and induction of thioredoxin reductase upon long term selenium supplementation in the rat

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Background: Selenium is an essential micronutrient for human and animals. Selenium treatment in supranutritional but subtoxic doses of 1 ppm and 5 ppm have shown to inhibit cell proliferation in both preneoplastic and neoplastic lesions in a rat liver carcinogenesis model. Selenium tumour prevention in chronic liver diseases requires long-term selenium supplementation and there is still quiet limited knowledge on selenium long term effects. Thioredoxin reductase (TrxR1) is a selenoenzyme essential for maintaining intracellular redox status and avoid oxidative stress. TrxR1 is overexpressed in proliferating liver nodules in the rat liver model. In this work we have studied selenium homeostasis in serum and liver as well as TrxR1 induction after long term selenium supplementation in the rat.
Materials and Methods: The kinetics of selenium uptake and accumulation and TrxR1 induction after treatment with sodium selenite in the drinking water in doses of 1 ppm and 5 ppm for 10 weeks have been studied in male Fisher rats. After withdrawal of selenium treatment the selenium status and TrxR1 induction were studied at 3 and 6 months of the experiment.
Results: Long term selenite exposure via the drinking water cause a dose dependent increase of blood and liver levels of selenium. This increase levels out at 6 weeks at the same level of selenium regardless of treatment and dose. Thus, there is no accumulation of selenium in blood and liver over time at chronic exposure. The same effect was seen on the induction of TrxR1 activity, while the induction of TrxR1 mRNA was only seen during the first two days of treatment. Discontinuation of selenite exposure did not result in